CHRONIC TOXICITY SUMMARY

ISOPHORONE

(1,1,3-trimethyl-3-cyclohexene-5-one; 3,5,5-trimethyl-2-cyclohexen-1-one; isoforon; isoacetophorone)

CAS Registry Number: 78-59-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**2,000** m**g/m^3** (400 ppb)
Critical effect(s)	Developmental effects (reduced crown-rump length of female rat fetuses); hepatocytomegaly and coagulative necrosis of the liver in mice
Hazard index target(s)	Development; liver

II. Chemical Property Summary (HSDB, 1995; CRC, 1994; CARB, 1997)

Description	Water-clear liquid with a peppermint-like odor
Molecular formula	$C_9H_{14}O$
Molecular weight	138.21 g/mol
Boiling point	215.2°C
Melting point	–8.1°C
Vapor pressure	0.44 torr at 25°C
Solubility	Slightly soluble in water (12,000 mg/L water at 25°C); miscible in organic solvents.
Conversion factor	5.65 µg/m^3 per ppb at 25°C

III. Major Uses and Sources

Isophorone is used extensively as a solvent in some printing inks, paints, lacquers, adhesives, vinyl resins, copolymers, coatings, finishes, and pesticides, in addition to being used as a chemical intermediate (HSDB, 1995). Since this compound has many different applications, release to the environment may originate from a wide variety of industrial sources including iron and steel manufacturers, manufacturers of photographic equipment and supplies, automobile tire plants, and printing operations. Coal-fired power plants may also emit isophorone to the air. Although it is mostly a man-made compound, isophorone has been found to occur naturally in cranberries (ATSDR, 1989). Occupational exposure may occur by inhalation or dermal contact. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2809 pounds of isophorone (CARB, 2000).

IV. Effects of Human Exposures

No information is available concerning long-term exposure or pharmacokinetics of isophorone in humans. In occupational monitoring studies, the time-weighted average concentration in breathing zones and workplace air of a screening plant ranged from 8.3-23 ppm and from 3.5-14.5 ppm, respectively (Samimi, 1982). Up to 25.7 ppm was detected in air of a silk screening printing plant in Pittsburgh, PA (Kominsky, 1983). The concentration in breathing zone samples from a decal manufacturing plant in Ridgefield, NJ was 0.7-14 ppm (Lee and Frederick, 1982). It was suspected that the reported eye and nose irritation of workers at the silk screening plant and at the decal manufacturing plant was the result of acute and subacute exposure to isophorone vapors.

Workers exposed to 5-8 ppm (28-45 mg/m^3) of isophorone for one month complained of fatigue and malaise (NIOSH, 1978). When concentrations were reduced to 1-4 ppm, no adverse effects were reported. Acute exposure studies in humans (up to 400 ppm for 1 to 4 minutes) resulted in eye, nose and throat irritation, nausea, headache, and dizziness or faintness (Union Carbide, 1963). Inhalation exposure for 15 minutes to 10 ppm isophorone produced only mild effects in human subjects while 25 ppm produced irritation to eyes, nose, and throat (Silverman *et al.*, 1946).

V. Effects of Animal Exposures

Few reports have been published regarding the pharmacokinetics of isophorone in experimental animals. Isophorone was widely distributed in the major organs of the rat following 4 hour inhalation exposure to 400 ppm (ATSDR, 1989). Oral gavage of 4000 mg/kg body wt to rats and a rabbit also resulted in wide distribution of the chemical. The highest blood levels of isophorone were reached by 30 min in rabbits following oral gavage and had decreased dramatically by 21 hours, indicating rapid absorption and elimination of the chemical. Preliminary results of a pharmacokinetic study indicate that rats treated orally with ^{14}C-isophorone excreted 93% of the radiolabel in the urine, expired air, and feces in 24 hours (ATSDR, 1989). The highest levels of ^{14}C-isophorone were found in the liver, kidney, preputial gland, testes, brain, and lungs. Several metabolites were identified in the urine of orally dosed rats and rabbits, including 3-carboxy-5,5-dimethyl-2-cyclohexene-1-one, 3,5,5-trimethylcyclohexanol, and some glucuronide conjugates (Dutertre-Catella *et al.*, 1978). A portion of the chemical was excreted unchanged in expired air.

In an early inhalation study, 10 Wistar rats/group and 10 guinea pigs/group, all of mixed sex, were exposed to 0, 25, 50, 100, 200, or 500 ppm isophorone 8 hr/day, 5 days/week for 6 weeks (Smyth *et al.*, 1942). Increased mortality and reduced body weights were observed at 100 ppm and up in both species. However, eye and nose irritation was noted only at the highest dose. Minor changes in blood chemistry and histopathological changes in the kidney and lungs were noted in treated animals. However, later investigations determined that the isophorone used in this study was contaminated with appreciable amounts of compounds (Rowe and Wolf, 1963).

Therefore, some of the adverse effects (i.e., the lung lesions) may have been due to the contaminants. The accuracy of the concentration data in the 1942 study is also questionable.

No treatment-related histopathological lesions were found in lungs, livers, or kidneys of male and female rats exposed intermittently (6 hr/day, 5 days/week) to 37 ppm isophorone for 4 weeks compared to controls (Hazleton Labs, 1968; summarized by ATSDR, 1989). Histological examination was limited to 30% of the control and treated rats. Body weight gain, mean absolute liver weights, and mean liver-to-body weight ratios of treated rats were significantly reduced compared to controls. Slight variations in hematological findings were noted in treated rats (increased lymphocytes and hemoglobin content; decreased neutrophils) but were not considered different from controls.

Rats (10/sex) were exposed to 500 ppm isophorone 6 hr/day, 5 days/week for up to 6 months (Dutertre-Catella, 1976; summarized by ATSDR, 1989). Irritation of eyes and nasal mucosa was observed. One female and three males in the treatment group died during the study, which was considered to be a treatment-related effect. But no exposure-related histopathological lung or liver lesions were observed compared to controls. Dutertre-Catella (1976) also exposed rats and rabbits (number per group per sex not stated) to 250 ppm isophorone 6 hr/day, 5 days/week for 18 months (Dutertre-Catella, 1976). Irritation of eyes and nasal mucosa was observed in both species, but no deaths occurred in the treatment groups. Histopathological examination of the lungs and kidneys, urinalysis, and hematological analysis revealed no exposure-related changes in either species. However, cytoplasmic microvacuolization of hepatocytes was observed in both species (ATSDR, 1989).

In a 90-day feeding study, 20 CFE albino rats/group/sex were given isophorone in their diet at concentrations of 0, 750, 1500, or 3000 ppm. Four beagle dogs/group/sex received isophorone in gelatin capsules at concentrations of 0, 35, 75, or 150 mg/kg body wt-day (AME, 1972a,b). High dose rats exhibited slightly reduced weight gain compared to controls (8-10%) for most of the study. Average weight gain among the exposure groups of beagle dogs remained essentially unchanged during the entire study. Urinalysis, hematology, and clinical chemistry indices found no treatment-related effects in the animals at either the interim or final toxicological examinations. Gross pathology and a limited histopathological examination observed no treatment-related effects in either species. Data on isophorone purity and possible loss of isophorone from rat diet due to vaporization were not presented.

In the most comprehensive isophorone toxicity study to date, 50 F344/N rats/group/sex and 50 B6C3F1 mice/group/sex were administered 0, 250 or 500 mg isophorone/kg body wt 5 days/week by oral gavage (in corn oil) for 103 weeks (Bucher *et al.*, 1986; NTP, 1986). Clinical signs of toxicity were not seen during the length of the study. However, several deaths in male and female rats at the high dose occurred early in the study. A steep decline in survival rate of high dose male rats occurred after week 90. Male and female rats and female mice in the high dose group exhibited only a slight decrease in body weight (<10%) compared to controls. A 13-week range finding study for the 2-year study did not find compound-related lesions in the kidney (or any other organs) of rats and mice exposed up to 1000 mg/kg body wt-day. However, pathological examination of rats exposed to isophorone for 2 years revealed non-neoplastic lesions in the kidney. Increased mineralization of the collecting ducts in isophorone-exposed

male (but not female) rats was observed. This lesion was characterized by basophilic aggregates of mineral most often found in the medullary collecting ducts and occurred coincidentally with lesions of chronic nephropathy. Nephropathy was observed in almost half the female controls and nearly all the male controls. Isophorone exposure appeared to increase both the severity of nephropathy in low dose male rats and the incidence of nephropathy in dosed female rats, but the effects were not pronounced. However, the isophorone potentiation of nephropathy in rats may be due to 'male rat-specific nephropathy' and may not have any relevance to human exposure (Strasser *et al.* 1988). Other adverse effects in kidneys of isophorone-treated male rats include tubular cell hyperplasia (in a dose-related manner) and epithelial hyperplasia of the renal pelvis. In mice, an increased incidence of chronic focal inflammation was observed in the kidneys of males, but was not considered treatment-related. A dose-dependent increase in fatty metamorphosis occurred in the adrenal cortex of male rats, but the biological significance of this change is unknown. All isophorone-exposed male mice had an increased incidence of hepatocytomegaly and coagulative necrosis of the liver. However, treatment-related liver lesions were not observed in female mice. Increased incidence of hyperkeratosis of the forestomach was observed in dosed male and high dose female mice, but was probably not a relevant treatment-related effect.

Published studies on possible reproductive effects of isophorone are lacking. An unpublished inhalation study conducted by a commercial laboratory (Bio/dynamics, 1984b) studied possible teratogenicity due to isophorone in rats or mice at inhaled doses up to 115 ppm. Groups of 22 female rats and 22 female mice were exposed to 0, 25, 50, or 115 ppm isophorone (6 hr/day) on gestational days 6-15. Maternal toxicity in rats included dose-dependent alopecia and cervical/anogenital staining. Low body weights (7-8%) were occasionally observed in the 115 ppm group. In mice, maternal toxicity was confined to slightly decreased weight (7-8%) on one day in the 115 ppm group. No significant differences were found in uterine implantations, fetal toxicity, and external and internal malformations among the animals. However, a slight, but significant, growth retardation in the form of decreased crown-rump length was present among the high dose fetal rats. Also, a slight, but insignificant, increase in extra ribs and/or rudimentary ribs was seen in rat and mouse fetuses at the highest dose. In a pilot study for this developmental toxicity investigation (12 females/species), exencephaly was observed in 1 rat and 1 mouse undergoing late reabsorption and in 2 live rat fetuses from dams exposed to 150 ppm isophorone on gestational days 6-15 (Bio/dynamics, 1984a). Exencephaly was not observed at any dose level in the primary study.

Dutertre-Catella (1976) did not find adverse reproductive or developmental effects in rats exposed to 500 ppm isophorone (6 hr/day, 5 days/week) for 3 months before mating and throughout gestation (females only) as well. The pups were not examined for internal malformations so the study was incomplete for determination of developmental effects.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Bio/dynamics 1984a,b
Study population	22 female mice/group, 22 female rats/group
Exposure method	Discontinuous whole body inhalation exposure during gestation (0, 25, 50, or 115 ppm)
Critical effects	Developmental effects (reduced crown-rump length of female rat fetuses); teratogenicity (exencephaly in fetal rats and mice) in range finding study at 150 ppm
LOAEL	115 ppm for reduced crown-rump length of female rat fetuses
NOAEL	50 ppm
Exposure continuity	6 hr/day during gestation
Exposure duration	Days 6-15 of gestation
Average experimental exposure	12.5 ppm (50 x 6/24)
Human equivalent concentration	12.5 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.4 ppm (400 ppb, 2 mg/m^3, 2,000 μg/m^3)

The inhalation study by Bio/dynamics (1984a,b) presents data that indicate exposure during gestation may be the most sensitive indicator of non-neoplastic toxicity by isophorone. Exposure of pregnant rats to 115 ppm isophorone during gestation resulted in significant growth retardation of female rat fetuses (reduced crown-rump length). Exposure to 50 ppm isophorone, the NOAEL, produced no developmental effects. The authors had removed the two shortest female fetuses prior to statistical analysis. The result was that there was no significant difference in fetal growth retardation; therefore, this adverse effect is not significant. However, this selective culling before the statistical analysis is not scientifically appropriate in this case. In addition, the authors did not perform some of the scheduled fetal examinations. Otherwise, the growth retardation might have had even greater statistical significance. The pilot study (Bio/dynamics, 1984a) observed exencephaly in a few mouse and rat fetuses at 150 ppm. Exencephaly was not considered significant by the authors because it was not present in any fetuses of the primary study (Bio/dynamics, 1984b). However, exencephaly is included as a critical effect in this summary because it is considered a serious teratogenic effect that was present at a dose (150 ppm) only slightly higher than the LOAEL of the primary study (115 ppm). Alopecia of adult female rats was observed in many of the exposed animals. However, this effect may be considered more of an acute dermal irritation than a chronic effect. In addition, cervical and anogenital staining seen in many exposed rats is not considered a chronic 'adverse' effect.

For comparison with the proposed REL of 0.4 ppm, the inhalation LOAEL of 250 ppm for mild liver effects (Dutertre-Catella, 1976) in rats and rabbits intermittently exposed to isophorone for 18 months was used to estimate a REL. Use of a time adjustment (6/24 x 5/7), an RGDR of 1, and a total UF of 100 (LOAEL to NOAEL = 3, interspecies = 3, and intraspecies = 10), also resulted in an estimated REL of 0.4 ppm. These results indicate that the REL will also protect against adverse liver effects.

While the toxicological significance of this liver effect observed by Dutertre-Catella (1976) is unknown, the NTP (1986) study observed an increased incidence of hepatocytomegaly and coagulative necrosis of the liver in treated male mice, but not in female mice and rats, orally gavaged with isophorone. Using 250 mg/kg-day as a LOAEL for mice and dividing by a total UF of 1000 (10 each for LOAEL to NOAEL, 10 for interspecies, and 10 for intraspecies) results in an oral REL of 0.25 mg/kg-day. Multiplying the oral REL by 3,500 $\mu g/m^3$ per mg/kg-day for route-to-route extrapolation results in a chronic inhalation REL estimate of 900 $\mu g/m^3$ (0.16 ppm), which is in good agreement with the REL developed from Dutertre-Catella (1976) and Biodynamics (1984a,b).

VII. Data Strengths and Limitations for Development of the REL

The strength of the database for isophorone is the consistent lack of relevant severe histopathological effects in the chronic inhalation study (Dutertre-Catella, 1976) and in the oral gavage study (NTP, 1986). Weaknesses of the database for isophorone include the lack of human exposure data, the lack of comprehensive long-term inhalation studies, and the lack of published peer-reviewed reproductive/developmental studies. The lack of human data may be due to isophorone's rather low potency for causing chronic, non-neoplastic, adverse effects. Inhalation of isophorone is a relevant route of exposure under occupational settings, but is most likely a minor route of exposure for the general population. Due to the insufficient characterization of the kidney and liver lesions in the oral gavage NTP study (Bucher *et al*, 1986; NTP, 1986) and the inhalation study (Dutertre-Catella, 1976), a comprehensive chronic study in rodent and non-rodent species would enhance the database for isophorone.

VIII. Potential for Differential Impacts on Children's Health

Since the REL is based on a developmental study, it is expected to be adequately protective of infants and children. However, there is no direct evidence in the literature to quantify a differential effect of isophorone in children relative to adults. Isophorone occurs in cranberries and thus presumably in cranberry juice, which is often mixed with other fruit juices. Children tend to consume more fruit juice. However, isophorone as a Hot Spot emission is unlikely to be a multimedia chemical, and there is no evidence to suggest that normal dietary levels of isophorone are associated with adverse health effects.

IX. References

AME. 1972a. Affiliated Medical Enterprises, Inc. 90-Day subchronic toxicity of isophorone in the rat (final report). Unpublished study performed by Affiliated Medical Enterprises, Inc. Princeton, NJ for Rohm and Haas Co. Philadelphia, PA. OTS 8d submission Doc. ID. 87812179, Microfiche No. 205975.

AME. 1972b. Affiliated Medical Enterprises, Inc. 90-Day subchronic toxicity of isophorone in the dog (final report). Unpublished study performed by Affiliated Medical Enterprises, Inc. Princeton, NJ for Rohm and Haas Co. Philadelphia, PA. OTS 8d submission Doc. ID. 87812178, Microfiche No. 205975.

ATSDR. 1989. Agency for Toxic Substances and Disease Registry. Toxicological profile for isophorone. U.S. Public Health Service. Atlanta, GA: ATSDR. PB90-180225.

Bio/dynamics. 1984a. Inhalation teratology probe study in rats and mice. Project No. 323771. Unpublished study performed by Bio/dynamics Inc. East Millstone, NJ. OTS Section 4 submission Doc. ID 40-8455042. Microfiche No. OTS0507219, pp. 1-33.

Bio/dynamics. 1984b. Inhalation teratology study in rats and mice. Final Report 3223772. Unpublished study performed by Bio/dynamics Inc. East Millstone, NJ for Exxon Biomedical Science, East Millstone NJ. OTS Section 4 submission Doc. ID 40-855049. Microfiche No. OTS 0507224, pp. 1-107.

Bucher JR, Huff J, and Kluwe WM. 1986. Toxicological and carcinogenesis studies of isophorone in F344 rats and B6C3F1 mice. Toxicology 39:207-219.

CARB. 1997. California Air Resources Board. Toxic Air Contaminant Identification List Summaries.

CARB. 2000. California Air Resources Board. California Emissions Inventory Development and Reporting System. (CEIDARS). Data from Data Base Year 1998. February 12, 2000.

CRC. 1994. CRC Handbook of Chemistry and Physics, 75th edition. Lide DR, ed. Boca Raton, FL: CRC Press Inc.

Dutertre-Catella H. 1976. Contribution to the analytical toxicological and bio-chemical study of isophorone (in French). Thesis for doctorate in pharmacology, Universite Rene Descartes, Paris. [Cited in Joint Assessment of Commodity Chemicals, No. 110, Isophorone, ECETOC, Brussels, 1989.]

Dutertre-Catella H, Nguyen PL, Dang Quoc Q, and Truhaut R. 1978. Metabolic transformations of the 3,5,5-2-cyclohexene-1-one trimethyl (isophorone). Toxicol. Eur. Res. 1(4):209-216.

Hazleton Labs. 1968. Assessment and comparison of subacute inhalation toxicities of three ketones. Final Report. Prepared by Hazleton Laboratories, Inc. Falls Church, VA for Exxon Chem Amers. Houston, TX. OTS 8d submission Doc ID. 878210935, Microfiche No. 206267.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (TOMES® CD-ROM Version). Denver, CO: Micromedex, Inc. (Edition expires 11/31/95).

Kominsky JR. 1983. Health hazard determination report no. HE 78-107-563. Pittsburgh, PA: Swinston Company.

Lee SA, and Frederick L. 1982. NIOSH health hazard evaluation report no. HHE80-103-827; NTIS PB82-189226.

NIOSH. 1978. National Institute for Occupational Safety and Health. Occupational exposure to ketones: criteria for a recommended standard. U.S. Department of Health, Education, and Welfare. DHEW (NIOSH) Publication No. 78-173.

NTP. 1986. National Toxicology Program. Toxicology and carcinogenesis studies of isophorone in F/344 rats and B6C3F_1 mice. NTP TR 291. NIH Publication No. 86-2547.

Rowe VK, and Wolf MA. 1963. Ketones. In: Industrial Hygiene and Toxicology, Second ed. Patty FA (ed.) New York: Interscience Publishers. p. 1764.

Samimi B. 1982. Exposure to isophorone and other organic solvents in a screen printing plant. Am. Ind. Hyg. Assoc. J. 43(1):43-48.

Silverman L, Schulte HF, and First MW. 1946. Further studies on sensory response to certain industrial solvent vapors. J. Ind. Hyg. Toxicol. 28(6):262-266.

Strasser J Jr, Charbonneau M, Borgoff SJ, Turner MJ, and Swenberg JA. 1988. Renal protein droplet formation in male Fischer 344 rats after isophorone (IPH) treatment. Toxicologist 8(1):136 (abstract).

Smyth HF Jr, Seaton J, and Fischer L. 1942. Response of guinea pigs and rats to repeated inhalation of vapors of mesityl oxide and isophorone. J. Ind. Hyg. Toxicol. 24(3):46-50.

Union Carbide Corporation. 1963. Toxicology Studies - Isophorone Summary Data Sheet. Industrial Medical Toxicology Dept. New York: Union Carbide.